

EXHIBIT A

Literature reference demonstrating unpredictability of specificity of synthetic peptide based antibodies (**emphasis added**):

Neuroscience, 2005;136(3):649-60.

Specificity of antibodies: unexpected cross-reactivity of antibodies directed against the excitatory amino acid transporter 3 (EAAT3).

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Source

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Abstract

Specific antibodies are essential tools for identifying individual proteins in biological samples. **While generation of antibodies is often straightforward, determination of the antibody specificity is not.** Here we illustrate this by describing the production and characterization of antibodies to excitatory amino acid transporter 3 (EAAT3). We **synthesized 13 peptides corresponding to parts of the EAAT3 sequence** and immunized 6 sheep and 30 rabbits. All sera were affinity purified against the relevant immobilized peptide. Antibodies to the peptides were obtained in almost all cases. Immunoblotting with tissue extracts from wild type and EAAT3 knockout animals revealed that **most of the antibodies did not recognize the native EAAT3 protein**, and that **some recognized other proteins**. Several immunization protocols were tried, but **strong reactions with EAAT3 were only seen with antibodies to the C-terminal peptides**. In contrast, good antibodies were obtained to **several parts** of EAAT2. EAAT3 was only detected in neurons. However, rabbits immunized with an EAAT3-peptide corresponding to residues 479-498 produced antibodies that labeled axoplasm and microtubules therein particularly strongly. **On blots, these antibodies recognized both EAAT3 and a slightly smaller, but far more abundant protein that turned out**

to be tubulin. The antibodies were fractionated on columns with immobilized tubulin. One fraction contained antibodies apparently specific for EAAT3 while **another fraction contained antibodies recognizing both EAAT3 and tubulin despite the lack of primary sequence identity between the two proteins.** Addition of free peptide to the incubation solution blocked immunostaining of both EAAT3 and tubulin. Conclusions: **Not all antibodies to synthetic peptides recognize the native protein.** The peptide sequence is more important than immunization protocol. **The specificity of an antibody is hard to predict because cross-reactivity can be specific and to unrelated molecules.** The antigen preabsorption test is of little value in testing the specificity of affinity purified antibodies.